

CLAIM AMENDMENT

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. **(Currently Amended)** A method for detecting an interaction between a first test protein and a second test protein at variable sensitivities via a detectable reporter gene, the method comprising:

providing a host cell wherein the host cell comprises a detectable reporter gene capable of expressing a detectable reporter gene product;

providing to the host cell a first hybrid protein comprising a polypeptide region capable of binding DNA and a bait polypeptide derived from the first test protein and a second hybrid protein comprising a polypeptide region capable of transcriptional activation and a prey polypeptide derived from the second test protein, wherein the host cell is additionally provided with the capacity to regulate the absolute or relative amounts of the first and second hybrid proteins;

regulating the amounts of the first and second hybrid proteins in a continuously adjustable manner so the detectable reporter gene is activated; and
determining the extent to which the detectable reporter gene has been activated whereby an interaction between the first test protein and the second test protein is detected,

wherein the first or second hybrid protein is provided by introducing into the host cell a first or second chimeric gene capable of being expressed in the host cell, wherein the first chimeric gene comprises a first exogenously activatable promoter, a sequence coding for a DNA binding region or polypeptide, and a sequence coding for the bait polypeptide,

wherein the first exogenously activatable promoter is activated by a first exogenous activator, and

wherein the first exogenous activator includes a natural or synthetic metabolically active or inactive steroid, steroid analog or steroid mimic.

Claims 2-9 (**Cancelled**)

10. (**Currently Amended**) A method for detecting an interaction between a first test protein and a second test protein at variable sensitivities via a detectable reporter gene, the method comprising:

- providing a host cell wherein the host cell comprises a detectable reporter gene capable of expressing a detectable reporter gene product;
- providing to the host cell a first hybrid protein comprising a polypeptide region capable of binding DNA and a bait polypeptide derived from the first test protein and a second hybrid protein comprising a polypeptide region capable of transcriptional activation and a prey polypeptide derived from the second test protein, wherein the host cell is additionally provided with the capacity to regulate the absolute or relative amounts of the first and second hybrid proteins;
- regulating the amounts of the first and second hybrid proteins in a continuously adjustable manner so the detectable reporter gene is activated; and
- determining the extent to which the detectable reporter gene has been activated whereby an interaction between the first test protein and the second test protein is detected,
- wherein the first or second hybrid protein is provided by introducing into the host cell a first or second chimeric gene capable of being expressed in the host cell,
- wherein the second chimeric gene comprises a second exogenously activatable promoter, a sequence coding for a transcriptional activation domain or polypeptide, and a sequence coding for the prey polypeptide,
- wherein the second exogenously activatable promoter is activated by a second exogenous activator, and
- wherein the second exogenous activators includes a natural or synthetic metabolically active or inactive steroids, steroid analog or steroid mimic.

11. (**Currently Amended**) The method of one of Claims 1 or 10 wherein at least one of the first or second exogenous activators is chosen from the group consisting of cortisol, hydrocortisone, estrogen, estradiol, estrone, progesterone, androgen, ecdysone,

retinoid, steroids which bind to orphan receptors, mineralocorticoid and mineralocorticoid analogues, and combinations thereof.

Claims 12-16 (**Cancelled**)

17. (**Currently Amended**) A method for detecting an interaction between a first test protein and a second test protein at variable sensitivities via a detectable reporter gene, the method comprising:

providing a host cell wherein the host cell comprises a detectable reporter gene capable of expressing a detectable reporter gene product;

providing to the host cell a first hybrid protein comprising a polypeptide region capable of binding DNA and a bait polypeptide derived from the first test protein and a second hybrid protein comprising a polypeptide region capable of transcriptional activation and a prey polypeptide derived from the second test protein, wherein the host cell is additionally provided with the capacity to regulate the absolute or relative amounts of the first and second hybrid proteins;

regulating the amounts of the first and second hybrid proteins in a continuously adjustable manner so the detectable reporter gene is activated; and

determining the extent to which the detectable reporter gene has been activated whereby an interaction between the first test protein and the second test protein is detected,

wherein the host cell is from a *Saccharomyces cerevisiae* strain comprising three integrated reporters for the detection of two-hybrid interactions, the first integrated reporter being a construct yielding a quantifiable product, the second and third integrated reporters being constructs yielding proteins sufficient to rescue nutrient auxotrophies,

wherein the first hybrid protein is provided by

introducing into the host cell a plasmid containing an ampicillin or kanamycin resistance gene, a colE1 origin of replication and a DNA sequence encoding a first hybrid protein comprising a bait polypeptide and a Gal4p DNA binding

domain, the expression of which is controlled by an integrated estrogen-inducible promoter; and
inducing expression of the first hybrid protein by incubating the host cell with an exogenous activator capable of activating the promoter; and
wherein the second hybrid protein is provided by
introducing into the host cell a plasmid containing an ampicillin or kanamycin resistance gene, a colE1 origin of replication and a DNA sequence encoding a second hybrid protein comprising a prey polypeptide derived from a library and the carboxyl-terminal end of the Gal 4p transcriptional activation domain, the expression of which is controlled by a rat glucocorticoid-inducible promoter; and
inducing expression of the second hybrid protein by incubating the host cell with an exogenous activator capable of activating the promoter.

Claims 18-21 (**Cancelled**)